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Epidermal Growth Factor Partially Restores Colonic Ion Transport Responses in Mouse Models of Chronic Colitis

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Background & Aims: Epidermal growth factor receptor (EGFR) activation, which plays an important role in regulating intestinal ion transport, can alleviate clinical symptoms such as diarrhea in patients with ulcerative colitis and promote mucosal restitution in animal models of colitis. Here, we investigate whether EGFR can regulate colonic ion transport in the setting of colitis. *Methods:* Distal colon from control mice and mice with colitis was retained for immunohistochemistry or mounted in Ussing chambers. Ion transport responses across the tissues to the calcium agonist carbachol and the adenosine 3′,5′-cyclic monophosphate agonist forskolin were measured with or without epidermal growth factor (EGF) pretreatment. *Results:* EGF pretreatment of normal colonic mucosa inhibited ion transport responses to carbachol and forskolin but potentiated the reduced ion transport responses seen in dextran sulfate sodium (DSS)-treated and mdr1a knockout mouse colon. Ion substitution studies and the sodium transport inhibitor amiloride showed that sodium movement primarily accounted for the potentiating effect of EGF in DSS-treated tissues, despite decreased sodium channel expression. EGF potentiation of transport responses in DSS-treated colon was completely blocked by the cytoskeletal disruptor cytochalasin D and the phosphatidylinositol 3-kinase inhibitor wortmannin, whereas the novel and conventional protein kinase C isoform inhibitor Gö6850 and the extracellular signal–regulated kinase inhibitor PD98059 partially reduced EGF effects. EGFR epithelial distribution and transforming growth factor α expression were also altered in DSS-treated tissues. *Conclusions:* Chronic inflammation uncovers a potentiating effect of EGFR activation on epithelial electrogenic sodium absorption that would be expected to ameliorate diarrheal symptoms associated with colitis.

The intestinal epithelium represents an important interface between the body and the external environment. The epithelium not only acts as a physical barrier between the lamina propria and the intestinal milieu but is also responsible for the active absorption and secretion of salt and water by the intestinal tract via the activity of ion transporters, exchangers, and selective ion channels.[1] Epithelial ion transport can be influenced by a variety of external factors, such as neurohumoral and inflammatory mediators, bacterial products or toxins, and paracrine factors.[1] The colon absorbs approximately 80% of the electrolyte-containing fluid that enters from the small intestine. Dysregulation of colonic epithelial ion transport, in particular excessive chloride secretion or inadequate sodium absorption, results in secretory diarrhea; this is a major symptom of a number of intestinal pathologies, including bacterial infection and inflammatory bowel disease (IBD).

A number of rodent models have been used to investigate the pathogenesis of IBD. One of the most commonly used models is the dextran sulfate sodium (DSS) model, which shares some similarity with human ulcerative colitis.[2] In this model of colitis, mice are administered DSS (3%–5%) in drinking water for 5–7 days, followed by 7 days on normal drinking water, to induce an acute form of colitis. Chronic colitis can be induced by administering several cycles of drinking water alternated with DSS.[2,3] A striking feature of this model and in tissues resected from patients with IBD is that despite the prominence of diarrhea as a symptom of IBD, colonic tissues from DSS-treated mice and patients with IBD exhibit reduced net ion transport responses to both Ca^{2+}- and 3′,5′-cyclic monophosphate (cAMP)-dependent chloride secretagogues as well as electrical nerve stimulation.[4–7] The mechanism(s) responsible for decreased ion transport responsiveness have not been fully elucidated, although a study by Sayer et al[7] provided evidence that decreased responses to the Ca^{2+}-dependent secretagogue carbachol (CCh), a stable analogue of the neurotransmitter acetylcholine, involve activation of a nicotinic cho-

Abbreviations used in this paper: CCh, carbachol; DSS, dextran sulfate sodium; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal–regulated kinase; Isc, short-circuit current; MAPK, mitogen-activated protein kinase; PI3-K, phosphatidylinositol 3-kinase; PKC, protein kinase C; TBST, Tris-buffered saline with 1% Tween 20; TGF, transforming growth factor.

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linergic receptor that is not apparent in the normal colon.

In addition to prosecretory pathways, integrated signaling mechanisms exist within intestinal epithelial cells that serve to limit chloride secretion. Previous work from our laboratory has shown that one of the critical negative regulators of Ca^{2+}-dependent chloride secretion is the epidermal growth factor receptor (EGFR).[8–11] Activation of the EGFR limits CCh-stimulated ion transport by mechanisms involving recruitment of phosphatidylinositol 3-kinase (PI3-K) and the extracellular signal–regulated kinase (ERK) isoforms of the mitogen-activated protein kinase (MAPK) family.[9,10] Therefore, EGFR has a role in acute regulation of epithelial function in addition to its well-established influence on cell growth and repair. Interestingly, a number of studies have investigated the role of EGFR in mucosal defense and repair after injury in models of colitis. Studies have shown that mice harboring a defective EGFR, known as waved-2 mice, or lacking the EGFR ligand transforming growth factor (TGF)-α, known as waved-1 mice, show increased susceptibility to DSS-induced colitis.[12,13] In addition, mice overexpressing TGF-α or those administered EGFR ligands before and during treatment with DSS show reduced susceptibility to DSS-induced colitis than seen in normal or untreated mice, respectively.[14,15] In summary, colonic tissues from colitic mice exhibit altered ion transport capability, and epidermal growth factor (EGF) has well-defined regulatory effects on ion transport in colonic epithelial cells in addition to its apparent protective effects on the colonic mucosa in colitis. Thus, the aim of this study was to investigate whether growth factor regulation of chloride secretion occurs in the setting of mucosal inflammation.

Materials and Methods

Induction of Colitis

Chronic colitis was induced in male Balb/c mice (8–10 weeks old) by administering 4% DSS for 5 treatment cycles. Each cycle consisted of 5 days on water containing DSS, followed by 7 days on normal drinking water. Control mice received normal drinking water. DSS-treated mice were killed at the completion of the fifth cycle of DSS administration. mdr1a$^{-/-}$ mice were obtained from a colony maintained at the University of California, San Diego, under conventional housing conditions. mdr1a$^{-/-}$ mice develop a spontaneous colitis featuring several classic clinical parameters such as decreased weight gain and diarrhea.[16] Distal colonic mucosa from 16-week-old male mdr1a$^{-/-}$ mice with confirmed colitis or from DSS-treated mice were used for electrophysiologic studies as described in the following text. Experiments were conducted with the approval of the Animal Subjects Committee at the University of California, San Diego.

Assessment of Colitis

Body weight was recorded after the completion of each treatment (DSS or normal water) in each cycle. At the completion of 5 treatment cycles, mice were killed by cervical dislocation and the colons removed. Colon length and wet weight, which serve as useful indicators of colitis,[7,17] were recorded for normal, DSS, and mdr1a$^{-/-}$ tissues. Pieces of distal colon were removed and fixed in 4% formalin, dehydrated through graded alcohols, and embedded in paraffin wax, and transverse sections were cut and stained with H&E. Colonic histology was assessed in DSS-treated and control tissues by a blinded, independent observer.

Colonic Epithelial Ion Transport Studies

A 3-cm segment of mid-distal colon was stripped of muscle layers and cut into smaller sections that were then mounted on specially designed Ussing chamber inserts with a window area of 0.07 cm^2. Tissues were bathed in oxygenated Ringer's solution at 37°C with the following composition (in mmol/L): 140 Na^+, 5.2 K^+, 1.2 Ca^{2+}, 0.8 Mg^{2+}, 120 Cl^-, 25 HCO_3^-, 2.4 $H_2PO_4^-$, 0.4 HPO_4^{2-}, and 10 glucose. The tissues were short circuited by an automated voltage clamp and the potential difference (PD), expressed in millivolts, across the tissues was monitored at intervals as an indication of net active ion transport. We found PD to be a more sensitive measure of ion transport events than short-circuit current (Isc), given the small dimensions of the tissues. Thus, ion transport responses were routinely expressed as changes in PD. However, identical conclusions were possible where Isc responses were measurable, as noted herein. Tissues were allowed to equilibrate for a 20-minute period, at which point baseline PD, Isc, and tissue conductance were measured before administration of any reagents. Ion substitution studies were performed using sodium isethionate, $CaSO_4$, and $MgSO_4$ in place of chloride, giving the Cl^--free Ringer's solution a final ionic concentration as follows (in mmol/L): 145 Na^+, 5.2 K^+, 1.2 Ca^{2+}, 2.4 SO_4, 1.2 Mg^{2+}, 120 isethionate, 25 HCO_3^-, 2.4 $H_2PO_4^-$, 0.4 HPO_4^{2-}, and 10 glucose. To prepare sodium-free Ringer's solution, choline (choline bicarbonate and choline chloride) was used as a substitute for sodium to give a final ionic concentration as follows (in mmol/L): 115 choline, 5.2 K^+, 1.2 Ca^{2+}, 1.2 Mg^{2+}, 119.8 Cl^-, 25 HCO_3^-, 2.4 $H_2PO_4^-$, 0.4 HPO_4^{2-}, and 10 glucose.

Immunohistochemical Staining

Sections of colonic tissues from normal and chronic DSS-treated mice were mounted on slides as described previously. Tissue sections were then deparaffinized in xylene and hydrated through a decreasing series of alcohols (100% to 95% to 70%). Endogenous peroxidase was masked with 0.3% hydrogen peroxide treatment for 30 minutes. Sections were then treated with 0.1% avidin and 0.01% biotin before overnight incubation with rabbit anti–α-ENaC (1:100) or rabbit anti-

EGFR (1:200). The secondary antibody, horseradish peroxidase–conjugated anti-rabbit (1:50), was added for 30 minutes. Slides were then washed in phosphate-buffered saline (3 times), developed with Nova red for 5–10 minutes, counterstained in Mayer's hematoxylin, and washed in H_2O. Rabbit serum was used as a negative control for nonspecific staining. For measurement of protein kinase activation in DSS-treated mouse distal colon following acute treatment with EGF, tissues were stripped of smooth muscle layers and placed on mounted gauze supports. Tissues were kept viable in oxygenated Ringer's solution maintained at 37°C. Tissues were then exposed basolaterally to either Ringer's solution as control or 16.7 nmol/L EGF for 20 minutes. The reaction was stopped by washing in ice-cold phosphate-buffered saline, and the tissues were then snap frozen in Tissue-Tek OCT (Sakura Finetek USA Inc, Torrance, CA). Sections were treated as previously described and stained with rabbit antiphospho-Akt1 (1:500) and horseradish peroxidase–conjugated anti-rabbit secondary antibody (1:100).

Western Blotting

Isolated distal colonic tissues were bathed in Ringer's solution, stripped of muscle layers, and placed in 0.5 mL of ice-cold lysis buffer (1% Triton X-100, 1 μg/mL leupeptin, 1 μg/mL pepstatin, 1 μg/mL antipain, 100 μg/mL phenylmethylsulfonyl fluoride, 1 mmol/L sodium vanadate, 1 mmol/L sodium fluoride, and 1 mmol/L EDTA in phosphate-buffered saline). Tissues were homogenized using a Tissue-Tearor (Biospec Products, Bartlesville, OK), and samples were centrifuged at 12,000 rpm for 10 minutes and the pellet was discarded. Samples were assayed for protein content (Bio-Rad protein assay kit; Bio-Rad, Hercules, CA) and adjusted so that each sample contained an equal amount of protein. A sample of lysate was mixed with an equal volume of 2× gel loading buffer (50 mmol/L Tris, pH 6.8, 2% sodium dodecyl sulfate, 200 mmol/L dithiothreitol, 40% glycerol, 0.2 bromophenol blue) and boiled for 4 minutes before separation by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Resolved proteins were transferred onto polyvinylidene membranes (NEN Life Science Products Inc, Boston, MA). After transfer, the membrane was preblocked with a 1% solution of blocking buffer (Upstate Biotechnology Inc, Lake Placid, NY) for 30 minutes followed by a 1-hour incubation with the appropriate concentration of primary antibody in 1% blocking buffer. After washing (5 × 10 minutes) in Tris-buffered saline with 1% Tween 20 (TBST), membranes were incubated for 30 minutes in horseradish peroxidase–conjugated secondary antibody (anti-mouse or anti-rabbit immunoglobulin G; Transduction Laboratories, Lexington, KY) in 1% blocking buffer. After washing in TBST (5 × 10 minutes), immunoreactive proteins were detected using an enhanced chemiluminescence detection kit (Roche Molecular Biochemicals, Indianapolis, IN). Densitometric analysis of Western blots was performed using NIH Image software.

Table 1. Macroscopic Parameters of DSS-Induced Colitis

	Control (n = 9)	DSS (n = 17)	*P* value (control vs DSS)
Body wt gain (*g*)	5.4 ± 1.8	1.9 ± 1.0	<.05
Colon length (*cm*)	9.5 ± 0.2	7.6 ± 0.1	<.0001
Colon wet wt (*g*)	0.24 ± 0	0.32 ± 0.01	<.0001

NOTE. Balb/c mice were treated with 4% DSS in drinking water for 5 cycles of 5 days on DSS followed by 7 days on normal drinking water. Control mice received normal drinking water for the same time frame. Body weight gain refers to the increase in body weight over the 5-cycle period. Colon length and wet weight were determined at the conclusion of the experimental period. Results are expressed as mean ± SEM. Statistical comparison was performed using Student *t* test.

Materials

DSS (mol wt, 36,000–50,000; ICN Biomedicals, Inc, Aurora, OH); amiloride, carbachol, and forskolin (Sigma Chemical Co, St. Louis, MO); wortmannin, PD98059, Gö6850, tetrodotoxin, and mouse anti–TGF-α (Calbiochem, San Diego, CA); rabbit polyclonal anti-EGFR (Santa Cruz Biotechnology, Santa Cruz, CA); and rabbit polyclonal antiphosphoAkt-1 (Upstate Biotechnology Inc) were obtained from the sources indicated. All other reagents were of analytical grade and were acquired commercially.

Statistical Analysis

Data are expressed as means ± SE. All comparisons between multiple treatment groups were performed by analysis of variance followed by the Student–Newman–Keuls posttest. Comparisons between data from paired groups of animals were performed using Student *t* test. All data were analyzed using GraphPad Instat version 3.0 (GraphPad Software Inc, San Diego, CA), with $P < .05$ considered significant.

Results

Assessment of Colitis Following Treatment With DSS

Over the course of induction of chronic DSS colitis, many of the mice exhibited classic macroscopic signs associated with murine colitis such as mottled fur and, on occasion, rectal bleeding. During the 60 days of treatment, comprising 5 DSS treatment cycles, DSS-treated mice gained significantly less weight than the paired control mice (Table 1; $P < .05$, n = 9). After the mice were killed, the colons from control and DSS-treated mice were removed and differences in length and wet weight determined. Colons from DSS-treated mice were significantly shorter than those from control mice ($P < .0001$) but were significantly heavier ($P < .0001$; Table 1), thus reproducing the pattern of acute DSS treatment effects on colon length and weight. Histologic damage was assessed in tissue sections stained with H&E. A moderate inflammatory infiltrate, comprised mostly of

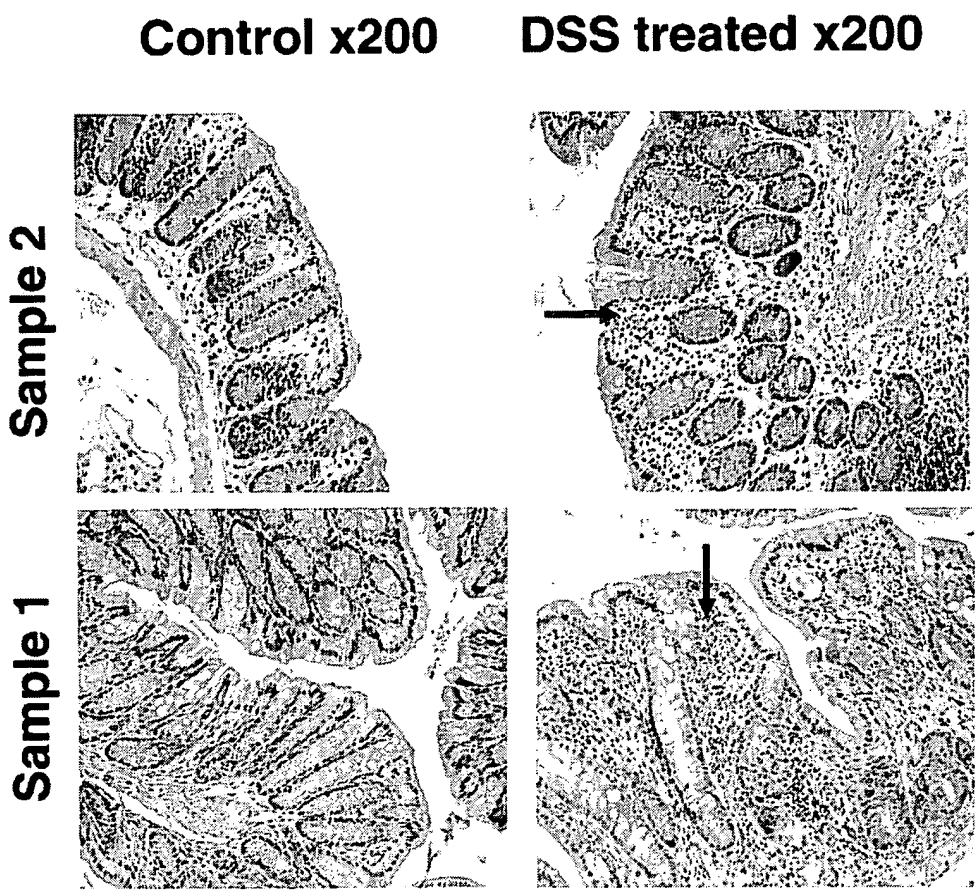

Figure 1. Chronic DSS treatment induces a mild colonic inflammation. Cross sections of distal mouse colon from normal and chronic DSS-treated mice were stained with H&E and viewed under light microscopy. No epithelial damage was observed. The *arrows* indicate increased mucosal infiltrate, which appears to consist mostly of lymphocytes, in colonic tissues from mice treated chronically with DSS.

lymphocytes, but with no overt damage to the epithelium, was observed in DSS tissues but not in controls (Figure 1).

Effect of EGF on Electrogenic Ion Transport Responses in Colitis

Our laboratory has previously shown that the EGFR plays an important role in regulating Cl^- secretory responses to both Ca^{2+}- and cAMP-dependent agonists in intestinal epithelial cells.[10,11,18] The principal ligands for the EGFR, EGF and TGF-α, have also been shown to have protective and restorative effects against chemical-induced colitis.[14,17,18] Consequently, we investigated whether EGFR regulation of electrogenic ion transport was perturbed in the setting of intestinal inflammation. CCh was used as a prototypic stimulus of Ca^{2+}-dependent Cl^- secretion. Serosal addition of CCh induced a sizeable change in PD across normal colon, and this response was significantly inhibited (66% ± 8%) by serosal pretreatment with EGF at a concentration that we have shown inhibits CCh-stimulated Cl^- secretion in colonic epithelial lines (16.7 nmol/L, 20 minutes, $P <$ .05, n = 7; Figure 2*A*). In tissues from chronic DSS-treated mice, CCh induced a small but reproducible change in PD. This effect was significantly enhanced by pretreatment with EGF ($P < .05$, n = 7; Figure 2*A*), thus showing that EGF and the EGFR differentially regulate Ca^{2+}-dependent ion transport in the setting of colitis compared with normal colon. Baseline conductance values in normal and DSS-treated colon were comparable (19.8 ± 1.5 vs 21.1 ± 1.8 mS · cm^{-2}). Treatment of normal colon with CCh induced similar changes in conductance whether or not EGF was present (1.19 ± 0.69 vs 0.54 ± 0.26 mS · cm^{-2}). Similarly, CCh treatment caused an equivalent change in conductance in inflamed tissue with or without EGF (0.71 ± 0.31 vs 0.54 ± 0.26 mS · cm^{-2}). EGF by itself had no significant effect on PD or conductance (data not shown). Thus, changes in PD evoked by CCh are likely reflective of net ion transport.

Having shown that EGF exerted opposing effects on ion transport responses to a Ca^{2+}-dependent agonist in

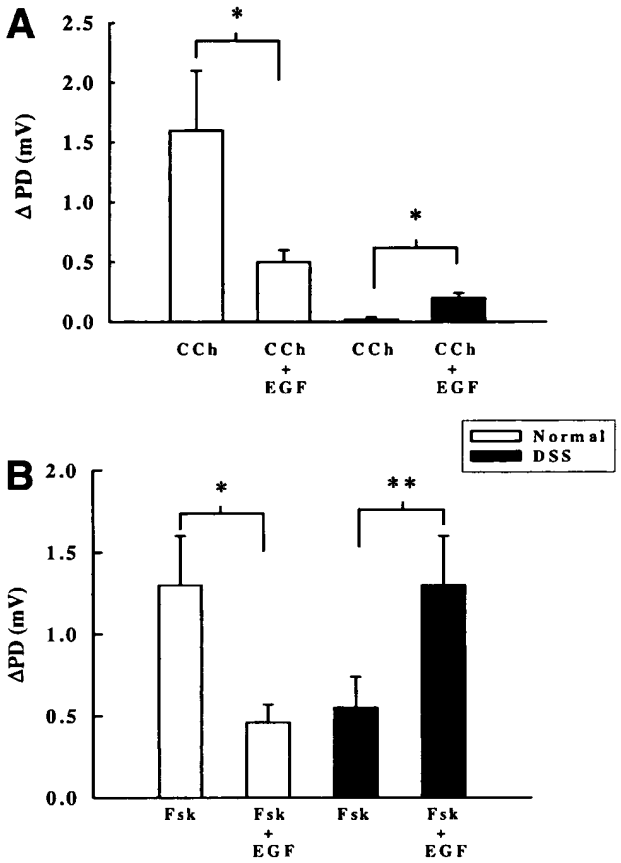

Figure 2. EGF differentially regulates ion transport responses in control versus DSS mouse colon. Colonic mucosae from normal (*white bars*) and chronic DSS-treated (*black bars*) Balb/c mice were stripped of underlying smooth muscle and mounted in Ussing chambers, and ion transport responses were assessed as changes in PD (ΔPD) following addition of (*A*) the Ca^{2+}-dependent agonist CCh or (*B*) the cAMP-dependent agonist forskolin. Tissues were pretreated with either serosal (basolateral) EGF (16.7 nmol/L) or vehicle control for 20 minutes before (*A*) serosal administration of CCh (100 μmol/L) or (*B*) bilateral administration of forskolin (10 μmol/L). Values are means ± SEM for (*A*) 7 or (*B*) 8 experiments. *Asterisks* denote significant effects of EGF; $*P < .05$, $**P < .01$ versus stimulus alone by Student *t* test.

normal versus chronic DSS-treated mouse colon, we next investigated the effects of pretreatment with EGF on ion transport responses to a cAMP-dependent agonist in the colonic mucosa of normal mice and mice with DSS-induced colitis. Pretreatment with EGF reduced ion transport responses to forskolin by 58% ± 12% ($P < .05$, n = 8) in normal mouse mucosa but potentiated the PD response to forskolin in the mucosa of mice with DSS-induced colitis by 2.5-fold ± 1-fold ($P < .01$, n = 8; Figure 2*B*). In normal mouse colon, the change in conductance following forskolin treatment was 2.3 ± 0.9 versus 1.97 ± 0.46 mS · cm^{-2} in the presence of EGF. In inflamed tissues, forskolin alone induced a change in conductance of 1.97 ± 0.46 versus 2.2 ± 0.99 mS · cm^{-2} after pretreatment with EGF.

To verify our data in the DSS colitis model, we reproduced our findings in a genetic model of colitis: the mdr1a$^{-/-}$ mouse model. This model is deficient in expression of the mdr1a glycoprotein and develops a spontaneous colitis from 5 to 8 weeks of age when conventionally housed.[19] In Figure 3, we observed that colonic mucosa from mdr1a$^{-/-}$ mice, stripped of muscle layers and mounted in Ussing chambers, exhibited small ion transport (PD) responses to forskolin (closed circles, Figure 3*A*). However, when tissues were treated basolaterally with EGF (16.7 nmol/L, 20 minutes; open circles), responses to forskolin were dramatically and significantly elevated ($P < .001$, n = 4). Peak Isc responses confirmed the potentiating effect of EGF on ion transport responses to forskolin (Figure 3*B*).

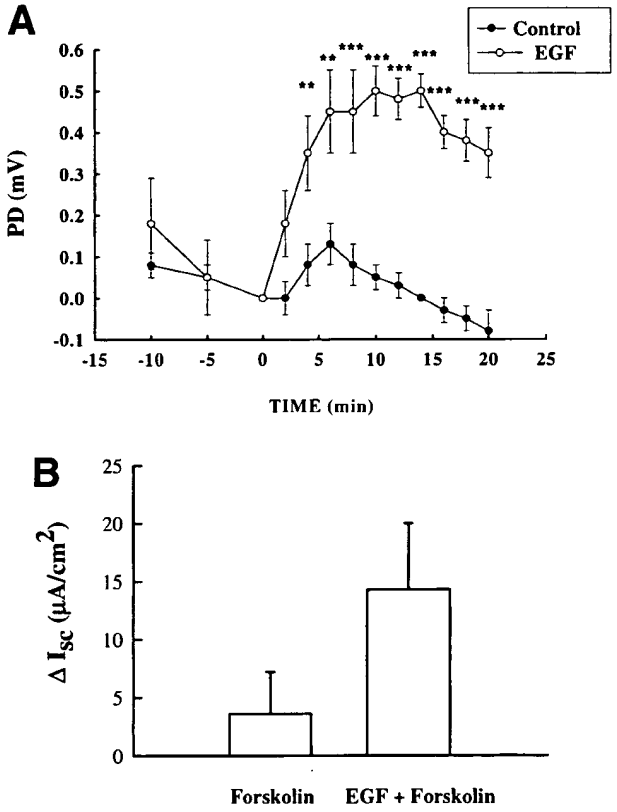

Figure 3. EGF increases ion transport responses in mdr1a$^{-/-}$ mouse colon. Colonic mucosae from mdr1a$^{-/-}$ mice were stripped of underlying smooth muscle and mounted in Ussing chambers. Tissues were pretreated with either serosal EGF (16.7 nmol/L) or vehicle control for 20 minutes before serosal administration of the cAMP-dependent agonist forskolin. Ion transport responses were assessed as (*A*) changes in PD (ΔPD in mV) or (*B*) changes in Isc (ΔIsc in μA/cm^2). Values are means ± SEM for 4 experiments. *Asterisks* denote significant effects of EGF; $**P < .01$, $***P < .001$ versus stimulus alone at individual time points by analysis of variance and Student–Newman–Keuls post-test.

Determination of the Charge-Carrying Ion Involved in EGF Potentiation of Electrogenic Ion Transport in DSS-Treated Mouse Colon

Because ion transport responses across DSS-treated mouse colon to the cAMP agonist forskolin were more robust than responses to CCh, we focused on forskolin-induced responses to define the precise transport process(es) underlying the potentiating effect of EGF. We thus conducted ion substitution studies to establish the nature of the charge-carrying ion involved in EGF-potentiated responses to forskolin. Physiologic solutions containing isethionate as a substitute for chloride, or choline in place of sodium, were used to bathe colonic mucosal tissues from normal and DSS-treated mice. In normal mouse colon, ion substitution studies showed that both chloride and sodium substitution reduced overall transport responses to forskolin ($P < .05$ [n = 4] and $P < .01$ [n = 5], respectively; Figure 4*A*). This suggests that forskolin may activate both chloride secretion and sodium absorption in normal tissues, although we cannot rule out an effect solely on chloride secretion because basolateral sodium is required for chloride uptake in the chloride secretory mechanism. Moreover, in the absence of either chloride or sodium, EGF was no longer able to reduce ion transport responses to forskolin, implying that the growth factor indeed alters active transport. Ion substitution studies in DSS-treated mucosal tissues showed that the reduced ion transport responses evoked by forskolin were also further attenuated in the absence of chloride or sodium, as seen in normal tissues. In contrast, the ability of EGF to potentiate the PD response to forskolin in inflamed tissues was essentially abolished in the absence of sodium, whereas removal of chloride ions did not significantly affect EGF potentiation of responses to forskolin (Figure 4*C*). Peak changes in Isc in normal (Figure 4*B*) and inflamed tissues (Figure 4*D*) showed a pattern very similar to the observed changes in PD. We conclude that EGF may selectively alter sodium transport in inflamed tissues.

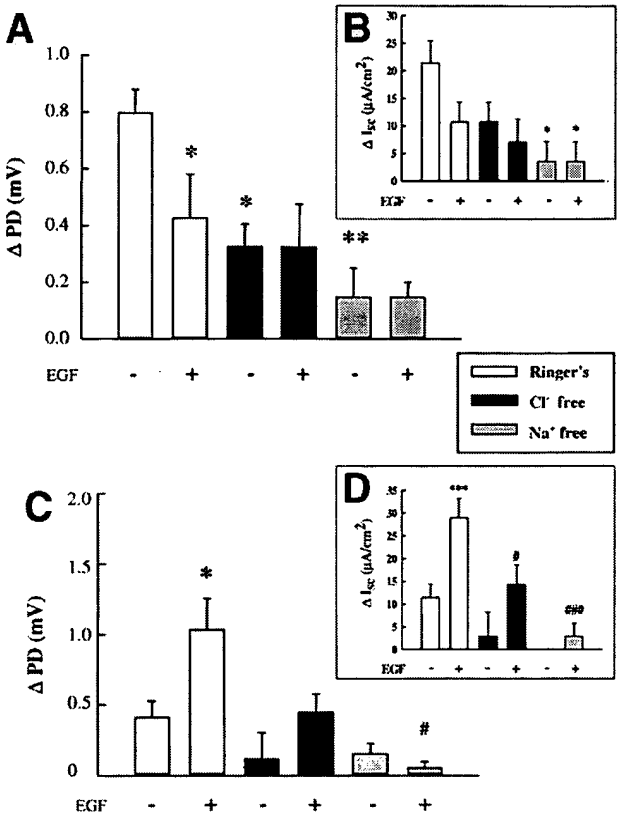

Figure 4. Sodium is the principal ion involved in EGF potentiation of ion transport responses to forskolin in DSS-treated colon. Ion substitution studies were performed on colonic mucosae by replacing chloride with isethionate or sodium with choline. Peak changes in PD in response to forskolin in the presence or absence of EGF were recorded in (*A*) normal (n = 4) or (*C*) inflamed (n = 5) colonic mucosal tissues and expressed as means ± SEM. Changes in Isc ($\mu A/cm^2$) are shown in *insets* (*B*, normal colon; *D*, inflamed colon). $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$ versus forskolin (complete Ringer's solution); $^{\#}P < .05$, $^{\#\#\#}P < .001$ versus EGF plus forskolin (complete Ringer's solution) by analysis of variance.

The Apical Sodium Channel ENaC Is Involved in EGF Potentiation of Ion Transport in Inflamed Mouse Colon

Having shown that sodium transport is likely critical for the inhibitory and potentiating effects of EGF on responses to forskolin in normal and colitic mouse colon, respectively, we further investigated these effects by determining whether the amiloride-sensitive apical sodium channel ENaC was involved in the responses observed in EGF-treated tissues. Preincubation of normal mouse colon with the ENaC inhibitor amiloride significantly reduced ion transport responses evoked by forskolin ($P < .05$, n = 4; Figure 5*A* and *B*). Furthermore, in the presence of amiloride, EGF had no further inhibitory effect on ion transport responses to forskolin. In colonic mucosal tissues from mice with chronic DSS-induced colitis, on the other hand, amiloride had no effect on ion transport responses evoked by forskolin alone (Figure 5*C* and *D*). Moreover, the ability of EGF to potentiate forskolin-stimulated changes in PD and Isc was completely blocked by preincubation with amiloride ($P < .05$, n = 4; Figure 5*C* and *D*). This implies that ENaC is a prominent target whereby EGF potentiates cAMP-dependent ion transport responses in inflamed colon.

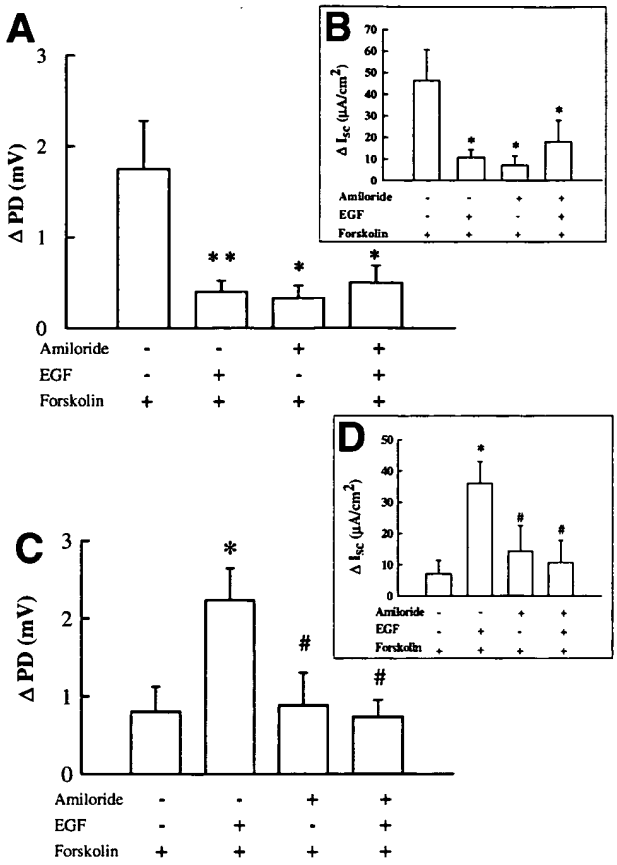

Figure 5. Increased ion transport responses to forskolin in EGF-treated DSS mouse colon are blocked by amiloride. The involvement of the apical sodium channel ENaC in the ability of EGF to potentiate forskolin-induced PD responses was investigated using the ENaC inhibitor amiloride. Colonic mucosae from (*A* and *B*) normal or (*C* and *D*) DSS-treated mice were treated apically with amiloride (50 μmol/L) for 30 minutes before the serosal addition of EGF (16.7 nmol/L) for 20 minutes and the subsequent bilateral administration of forskolin (10 μmol/L). Ion transport responses were measured as (*A* and *C*) changes in PD (in mV) or (*B* and *D*) Isc (in $\mu A/cm^2$) and expressed as means ± SEM for 4 experiments. $^*P < .05$, $^{**}P < .01$ versus forskolin alone; $^{\#}P < .05$ versus EGF plus forskolin by analysis of variance.

Immunostaining of Tissues for the Apical Sodium Channel Subunit α-ENaC

Colonic mucosae from humans with IBD and murine models of colitis exhibit decreased levels of overall ion transport. Electrophysiologic studies have shown that both electrogenic chloride secretory responses and electrogenic sodium absorption are suppressed in colitis.[7,12,20] In addition, activity and expression of ion transporter proteins such as Na^+,K^+–adenosine triphosphatase are decreased in both human IBD tissues and in models of inflammation.[4,21–23] Because decreased sodium absorption has been shown to be one of the major factors contributing to the diarrhea associated with colitis, we investigated whether expression of the apical sodium channel in colonic epithelial cells was affected by long-term treatment with DSS. The α-ENaC subunit represents the basic building block of the apical Na^+ channel and, in addition to forming a heterotrimeric structure with the β and γ subunits, it also contains the amiloride binding site. Staining of cross sections from control and DSS-treated colonic tissues with a rabbit antibody against α-ENaC (courtesy of Dr Dale J. Benos, University of Alabama at Birmingham) showed that tissues from DSS-treated mice exhibited decreased levels of α-ENaC compared with control colon (Figure 6).

An Intact Cytoskeleton Is Necessary for the Potentiating Effect of EGF on Ion Transport Responses in Inflamed Mouse Colon

The actin cytoskeleton has been shown in previous studies to participate in ion transport responses evoked by forskolin across cultured colonic epithelial cells.[24–26] Therefore, we next investigated whether cytoskeletal integrity was required for the modulating effects of EGF on ion transport responses in normal and colitic mouse colon. Preincubation of colonic mucosa from normal mice with the cytoskeletal disruptor cytochalasin D significantly inhibited ion transport responses to forskolin ($P < .05$ and $P < .01$, n = 4; Figure 7*A* and *B*). Treatment of cytochalasin D–pretreated tissues with EGF was unable to produce any further inhibitory effect on forskolin-induced ion transport. Likewise, cytochalasin D pretreatment of tissues from mice with DSS-induced colitis abolished the potentiating effect of EGF on forskolin-induced PD and Isc responses ($P < .05$, n = 4; Figure 7*C* and *D*). Changes in Isc responses to forskolin in inflamed tissues treated with cytochalasin D (Figure 7*D*) were below the level of detection, but tissue conductance was not significantly affected by cytochalasin D (data not shown), validating PD as a measure of net transport. These data indicate that an intact cytoskeleton is required for EGF to potentiate ion transport responses to forskolin in inflamed colonic mucosa.

The Potentiating Effect of EGF on cAMP-Dependent Ion Transport Responses in Inflamed Mouse Colon Is Mediated by ERK, PI3-K, and Protein Kinase C Activation

EGFR activation principally engages one of 2 signaling pathways to mediate regulatory effects on ion transport in cultured epithelial cells, via either ERK or PI3-K activation.[9,10] Consequently, we next investigated if either of these signaling intermediates was involved in the potentiating effect of EGF on forskolin-stimulated ion transport in inflamed mouse colon. MEK1 is the

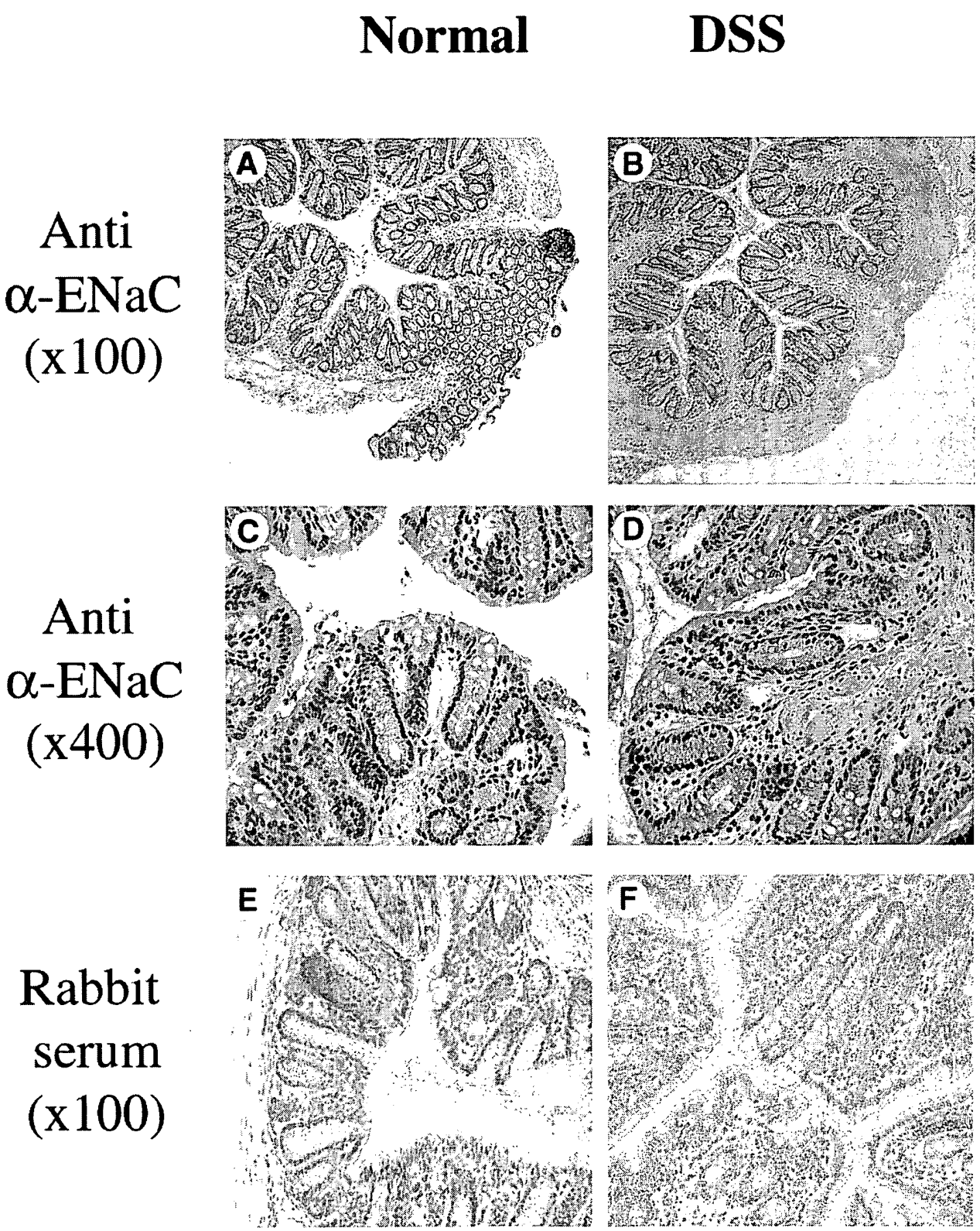

Figure 6. α-ENaC expression is decreased in inflamed distal mouse colon. Immunostaining for the α subunit of the apical sodium channel ENaC in colonic epithelium from (*A* and *C*) normal and (*B* and *D*) DSS-treated mice shows that this protein is highly expressed in surface epithelial cells in control tissues but exhibits decreased expression in colonic epithelium in DSS-treated mice. Rabbit serum was used as a negative control for nonspecific staining of (*E*) normal and (*F*) DSS-treated colon. The staining shown is representative of 5 different samples.

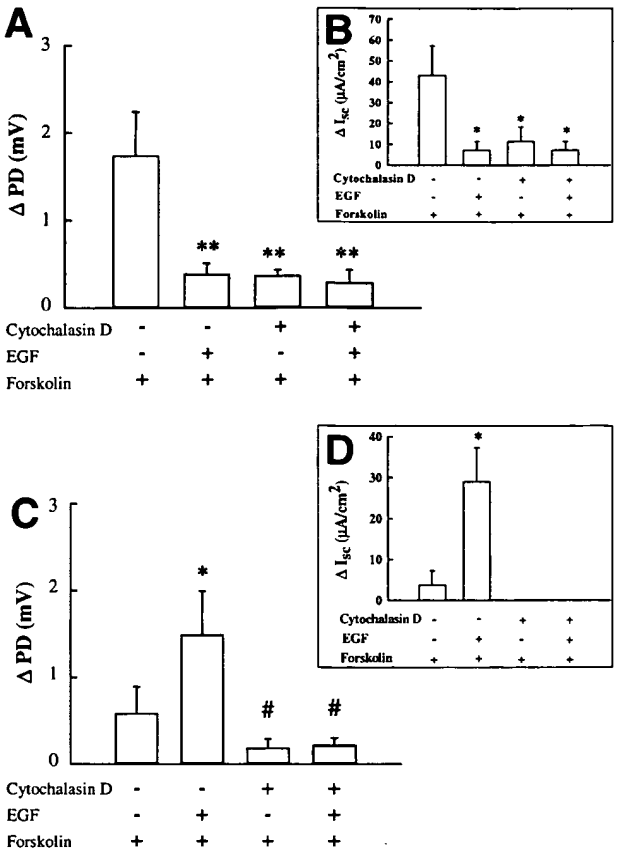

Figure 7. EGF potentiation of cAMP-stimulated ion transport in inflamed colon is dependent on the actin cytoskeleton. The role of the actin cytoskeleton in EGF potentiation of forskolin ion transport responses was investigated using the cytoskeletal disruptor cytochalasin D. Colonic mucosal segments from (*A* and *B*) normal or (*C* and *D*) DSS-treated mice were treated bilaterally with either cytochalasin D (20 μmol/L) or Ringer's solution for 30 minutes before serosal addition of EGF (16.7 nmol/L). Tissues were then treated bilaterally with forskolin (10 μmol/L). Ion transport responses were measured as changes in (*A* and *C*) PD (in mV) or (*B* and *D*) Isc (in μA/cm²) and are expressed as means ± SEM for 4 experiments. $^{*}P < .05$, $^{**}P < .01$ versus forskolin alone; $^{\#}P < .05$ versus EGF plus forskolin by analysis of variance.

enzyme in the Ras-ERK cascade that is responsible for activating ERK1/2. Inhibition of MEK1 with PD98059 prevents activation of ERK. In normal tissues, PD98059 had no effect on the ability of EGF to reduce ion transport responses to forskolin (Figure 8*A* and *B*), and indeed PD98059 reduced forskolin-stimulated ion transport by itself. These data are in keeping with prior observations that ERK1/2-dependent pathways are more frequently recruited to modify chloride secretion when EGFR is "transactivated" by G-protein–coupled receptor ligands rather than by its cognate ligand.[10,11] In contrast, PD98059 pretreatment of colonic mucosa from DSS-treated mice partially reduced EGF potentiation of forskolin-induced PD and Isc responses and also delayed the kinetics of the response ($P < .05$, n = 3; Figure 8*C* and *D*). PD98059 had no significant effect on the ion transport response to forskolin in inflamed tissues in the absence of EGF. Our data indicate that EGF potentiates forskolin-induced ion transport at least in part via activation of the ERK isoforms of the MAPK family.

The other major signaling pathway used by EGF in ion transport regulation is the PI3-K pathway. Treatment of normal distal colonic mucosa with the PI3-K inhibitor wortmannin did not reverse EGF-mediated inhibition of ion transport responses to forskolin but did reduce forskolin-stimulated ion transport by itself ($P < .05$ and $P < .01$, n = 3; Figure 9*A* and *B*). On the other hand, pretreatment of colonic mucosal tissues from DSS-treated mice with wortmannin completely blocked the ability of EGF to potentiate forskolin-stimulated ion

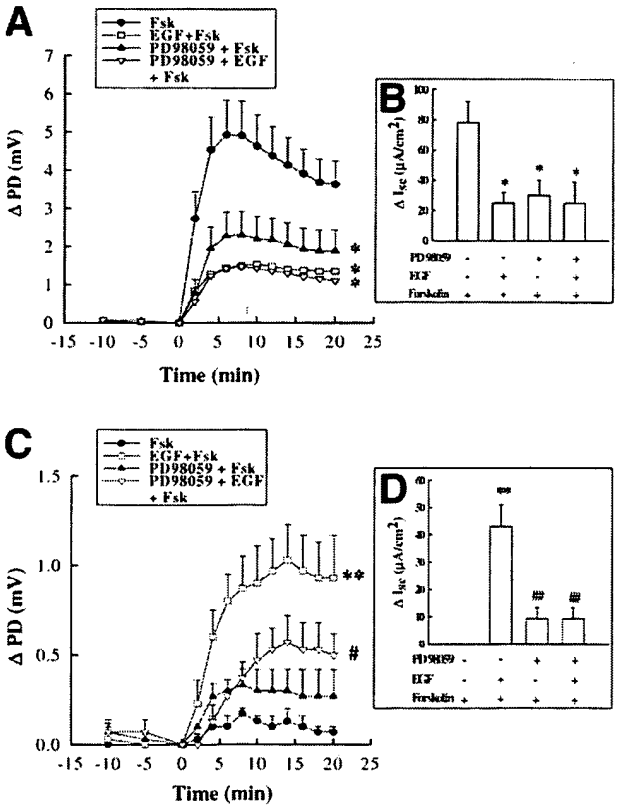

Figure 8. ERK activation is required for EGF regulation of ion transport in inflamed but not uninflamed mouse colon. Involvement of ERK1/2 in the regulatory effects of EGF (16.7 nmol/L) on forskolin-stimulated ion transport responses in normal or inflamed mouse colon was investigated using the MEK1 inhibitor PD98059. PD98059 (50 μmol/L) was administered bilaterally to (*A* and *B*) normal and (*C* and *D*) DSS-treated mouse colonic mucosae for 30 minutes before addition of EGF and subsequent stimulation with forskolin (10 μmol/L). Ion transport responses were measured as changes in PD or Isc and are expressed as means ± SEM for (*A* and *B*) 4 or (*C* and *D*) 3 experiments. $^{*}P < .05$, $^{**}P < .01$ versus forskolin alone; $^{\#}P < .05$, $^{\#\#}P < .01$ versus EGF plus forskolin by analysis of variance.

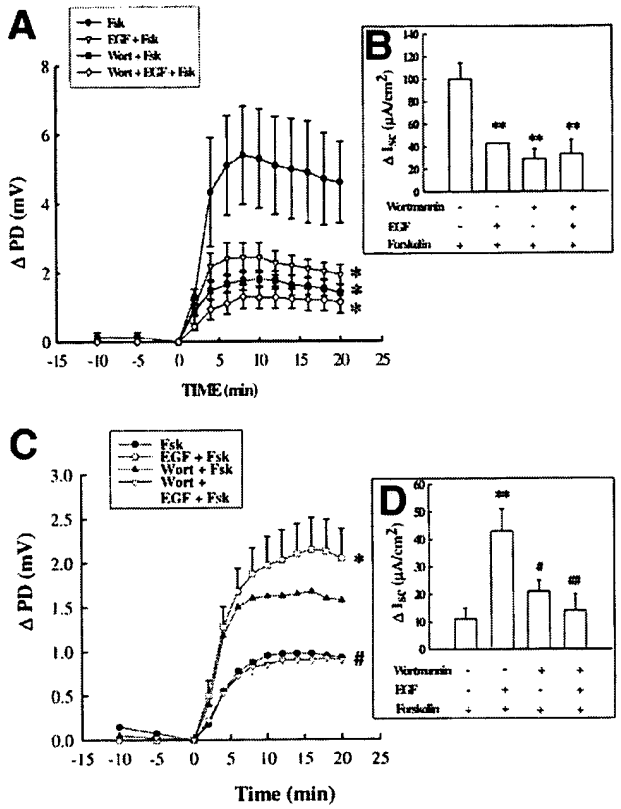

Figure 9. PI3-K activation mediates EGF potentiation of ion transport responses in DSS-treated mouse colon. Colonic mucosae from (*A* and *B*) normal and (*C* and *D*) chronic DSS-treated mice, stripped of underlying smooth muscle and mounted in Ussing chambers, were incubated bilaterally with either the PI3-K inhibitor wortmannin (50 nmol/L) or vehicle control for 30 minutes before administration of EGF (16.7 nmol/L) or Ringer's solution for 20 minutes. Tissues were then stimulated bilaterally with forskolin (10 μmol/L). Responses were measured as changes in PD or Isc and are expressed as means ± SEM for (*A* and *B*) 3 and (*C* and *D*) 4 experiments. $*P < .05$, $**P < .01$ versus forskolin alone; $\#P < .05$ versus EGF plus forskolin. Statistical differences were assessed by analysis of variance.

transport responses ($P < .05$ and $P < .01$, n = 4; Figure 9*C* and *D*). These data indicate that activation of PI3-K mediates the potentiating effect of EGF on forskolin-stimulated sodium absorption in the setting of inflammation but does not appear to be involved in EGF-mediated inhibition of forskolin responses in normal distal colon.

Likewise, EGFR activation in colonic epithelial cells has been shown to lead to mobilization of protein kinase C (PKC) isoforms.[27–29] In fact, several studies have shown a role for PKC isoforms in the regulation of intestinal ion transport,[30,31] and our laboratory has previously shown that PKCε activation is involved in the inhibitory effect of EGF on Ca^{2+}-dependent chloride secretion in cultured T_{84} cells.[27] Finally, therefore, we investigated whether PKC activity played a role in the ability of EGF to stimulate sodium absorption in inflamed colon. The novel and conventional PKC isoform inhibitor Gö6850, administered to normal mouse colon, caused a partial reversal of EGF-mediated inhibition of forskolin-stimulated ion transport ($P < .01$ and $P < .05$ vs forskolin, n = 4; Figure 10*A* and *B*) compared with EGF inhibition of the ion transport response to forskolin ($P < .05$ and $P < .001$ vs forskolin alone). Gö6850 by itself significantly inhibited forskolin-stimulated ion transport ($P < .01$ and $P < .001$, n = 4). Pretreatment of DSS-treated mouse mucosal tissues with Gö6850 before EGF treatment also partially inhibited EGF potentiation of ion transport responses to forskolin ($P < .05$, n = 7; Figure 10*C* and *D*). These data indicate that PKC isoforms are also involved in the altered ion transport responses to forskolin seen in EGF-treated mouse colon.

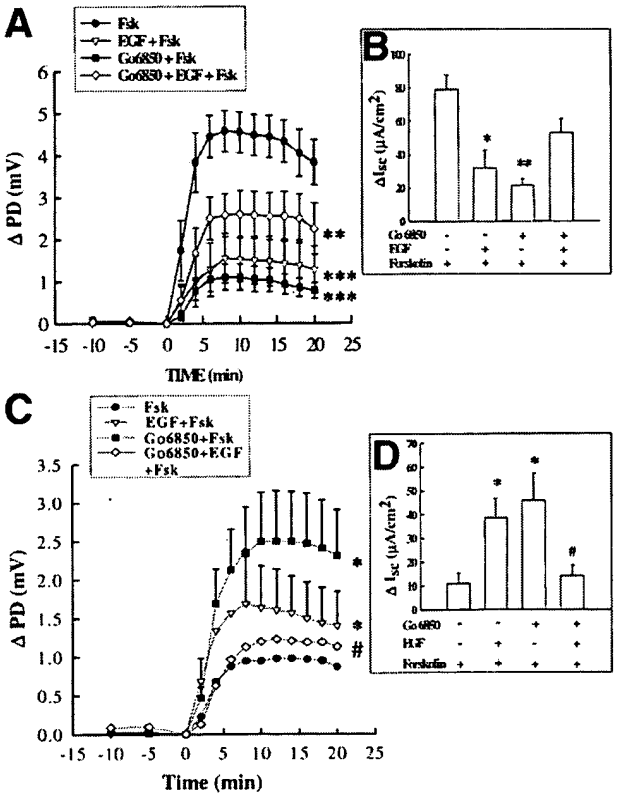

Figure 10. Novel and conventional PKC isoforms are involved in the ability of EGF to potentiate ion transport responses in DSS-treated mouse colon. Colonic mucosae from chronic DSS-treated mice, stripped of underlying smooth muscle and mounted in Ussing chambers, were incubated bilaterally with either the novel and conventional PKC isoform inhibitor Gö6850 (50 nmol/L) or vehicle control for 30 minutes before administration of EGF (16.7 nmol/L) or Ringer's solution for 20 minutes. Tissues were then stimulated bilaterally with forskolin (10 μmol/L). Responses were measured as changes in (*A* and *C*) PD or (*B* and *D*) Isc and are expressed as means ± SEM for (*A* and *B*) 4 and (*C* and *D*) 7 experiments. $*P < .05$ versus forskolin, $\#P < .05$ versus EGF plus forskolin. Statistical differences were assessed by analysis of variance.

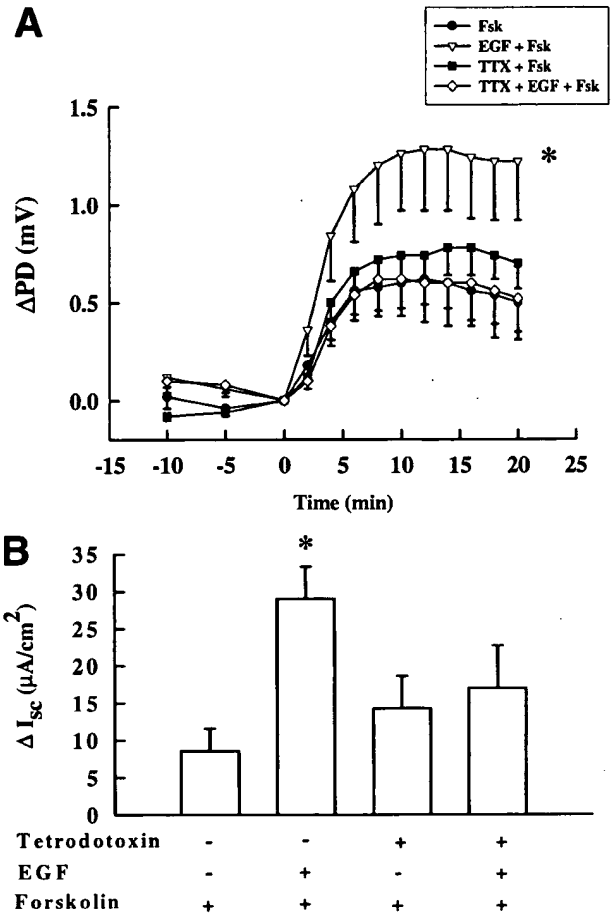

Figure 11. Role of lamina propria neurons in EGF potentiation of forskolin-stimulated ion transport. Colonic mucosae from DSS-treated mice, stripped of underlying smooth muscle and mounted in Ussing chambers, were incubated basolaterally with the neuronal inhibitor tetrodotoxin (1 μmol/L) before administration of EGF (16.7 nmol/L) followed by forskolin or forskolin alone (10 μmol/L). Changes in ion transport were measured as changes in (*A*) PD or (*B*) Isc. $*P < .05$ versus forskolin alone; n = 5.

Effect of Tetrodotoxin on EGF Potentiation of Forskolin-Stimulated Ion Transport in DSS-Induced Colitis

Because we administered EGF to the serosal side of the colonic mucosal tissues mounted in Ussing chambers, we next investigated whether subepithelial elements were involved in EGF potentiation of forskolin-stimulated ion transport. Serosal preincubation with the neuronal blocker tetrodotoxin (1 μmol/L) appears to inhibit, at least in part, the potentiating effect of EGF on forskolin-stimulated ion transport (n = 5; Figure 11*A* and *B*). In normal colon, tetrodotoxin had no effect on responses to either forskolin alone or on EGF inhibition of forskolin-stimulated ion transport (data not shown). These data suggest that the ability of EGF to potentiate ion transport responses to forskolin in inflamed colon is partly dependent on subepithelial neurons.

EGF Activates Epithelial Signaling Pathways in Inflamed Mouse Colon

We next investigated whether serosal EGF treatment of inflamed mouse colon could directly activate epithelial signaling pathways that lie downstream of the EGFR that are known to be involved in epithelial ion transport regulation. Colonic mucosae from DSS-treated mice were stripped of underlying smooth muscle layers and placed on a gauze platform, serosal side up. Mucosal and serosal surfaces were exposed to oxygenated physiologic Ringer's solution at 37°C. Paired tissues were treated with either serosal EGF (16.7 nmol/L) or Ringer's solution for 20 minutes. Tissues were rinsed in ice-cold Ringer's solution and then snap frozen in OCT. Tissue sections were stained for Akt phosphorylation as a marker of PI3-K activation. Figure 12 shows representative staining for phospho-Akt in colonic tissues treated with Ringer's solution (Figure 12*A* and *C*) or EGF (Figure 12*B* and *D*), taken from 5 fields of view in 5 separate experiments. Akt phosphorylation (brown staining) was confined to the epithelial layers and is indicated by the arrows. Visual assessment of staining intensity by a blinded observer indicated higher epithelial phosphorylated Akt staining intensity in EGF-treated versus untreated inflamed mouse colonic mucosa (n = 5).

We also investigated whether the potentiating effect of EGF was facilitated by a change in expression levels of the EGFR in the setting of inflammation. Immunohistochemical staining for the EGFR indicated a possible increase in EGFR expression levels in inflamed versus control mouse colon. There did seem to be a definite increase in the intensity of epithelial EGFR staining in colonic crypts from DSS-treated mice, whereas EGFR staining in control tissues was more pronounced in surface epithelial cells (Figure 13*A*). This suggests a possible redistribution of the EGFR in inflammation. Interestingly, expression of the major EGFR ligand in the intestine, TGF-α, was significantly increased as measured by densitometric analysis of TGF-α Western blots ($P < .05$ vs control, n = 10; Figure 13*B* and *D*).

Discussion

Although the etiology of ulcerative and Crohn's colitis is unclear, it is apparent that dysregulation of normal epithelial ion transport processes, decreased expression of specific components of the sodium- and chloride-transporting machinery of epithelial cells, and reduced effectiveness of the gut barrier clearly contribute

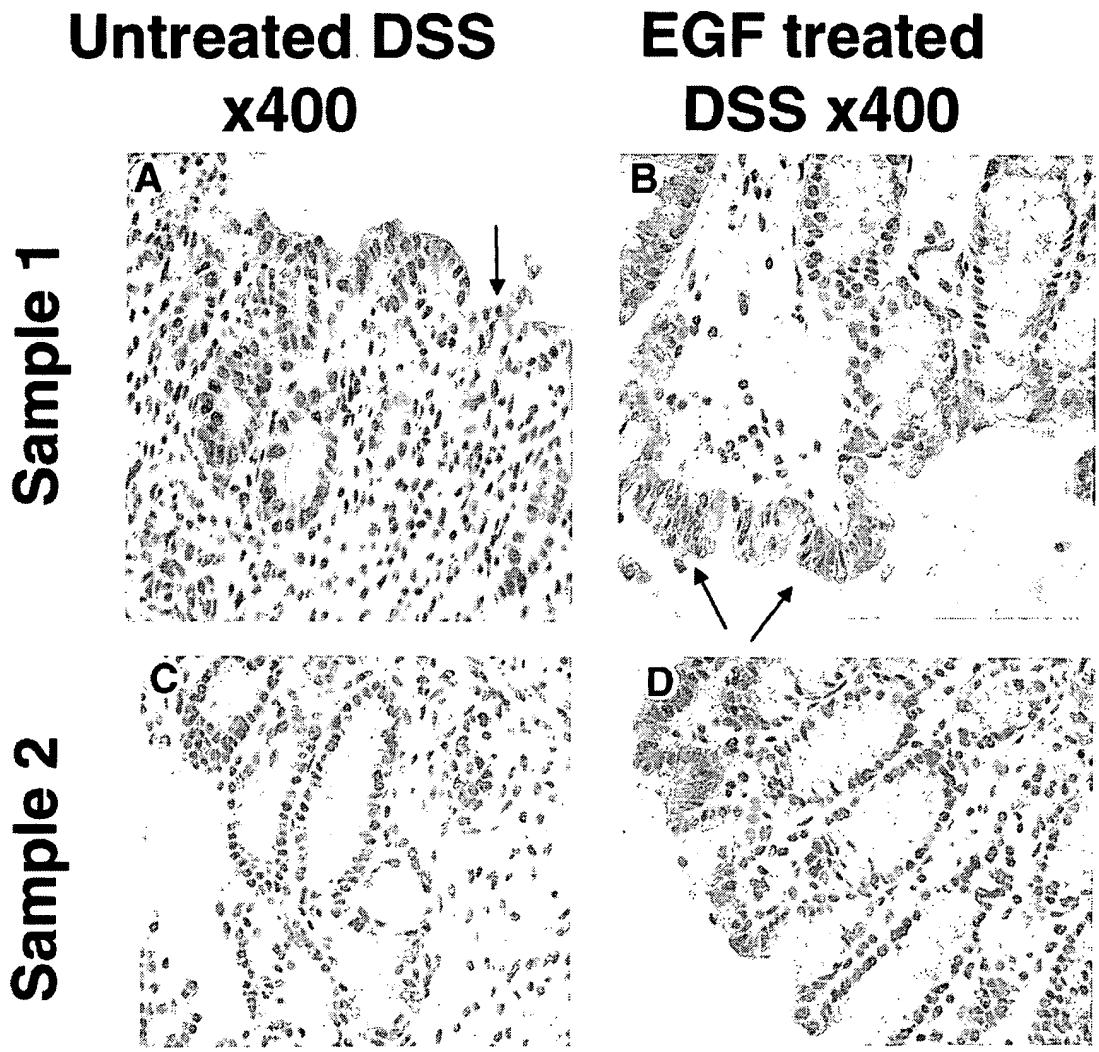

Figure 12. EGF activates epithelial PI3-K signaling in inflamed mouse colon. Paired segments of stripped colonic mucosa from DSS-treated mice were treated with either EGF (16.7 nmol/L) or Ringer's solution for 20 minutes and then snap frozen. Tissue sections were stained for phosphorylation of the PI3-K downstream molecule Akt-1. *Arrows* indicate phosphorylated Akt-1 (brown staining) in (*A* and *C*) Ringer's-treated DSS colon and (*B* and *D*) EGF-treated tissue. Samples are representative of 5 individual fields of view for 5 separate experiments. (Original magnification 400×.)

to both diarrhea and chronic inflammation associated with colitis. In this study, we have shown that ion transport responses to both Ca^{2+}- and cAMP-dependent agonists are diminished in the distal colon from mice with chronic DSS-induced colitis, mirroring previous studies using the acute DSS model.[5–7] However, we have extended this area of investigation by showing that prior administration of EGF to the serosal side of inflamed colonic tissue can potentiate ion transport responses to both CCh and forskolin, thereby tending to normalize function. In contrast, pretreatment with EGF reduces ion transport responses to both of these stimuli in normal colonic tissues, thus indicating that EGF and the EGFR behave differently in the setting of inflammation. These findings were further supported by data obtained from the spontaneous mdr1a$^{-/-}$ mouse colitis model, which showed that EGF could also potentiate ion transport responses to forskolin in colonic mucosae isolated from this model. EGF has been used prophylactically to limit the extent of mucosal inflammation caused by chemical models of inflammation in rodent studies.[15,32] In addition, recent clinical evidence indicates that EGF enemas can decrease the inflammation and diarrhea associated with ulcerative colitis.[33] Due to its wound healing and restitutive effects, EGF and other EGFR ligands, such as TGF-α, whose expression is increased in both experimental and human colitis,[34,35] may act in a number of ways to restore normal colonic integrity and function.

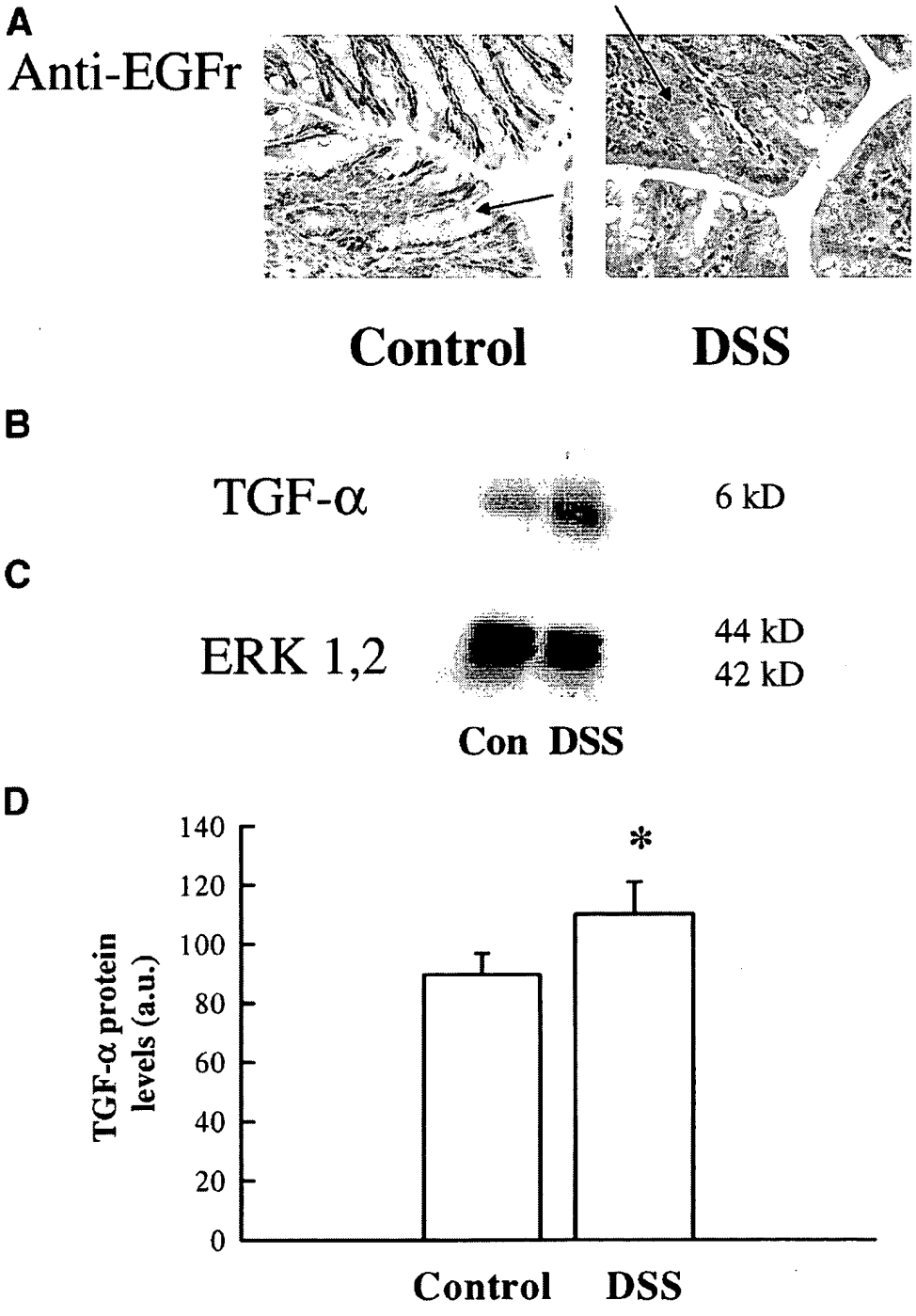

Figure 13. DSS-induced chronic colitis alters epithelial EGFR distribution and increases mouse colonic TGF-α expression. (*A*) Immunohistochemical staining for the EGFR (*arrows*) indicated increased EGFR expression in crypt epithelial cells in DSS-treated mouse colon versus control mouse colonic expression. Colonic mucosae from normal and DSS-treated mice were stripped of muscle layers and lysed, and expression of TGF-α was measured by Western blotting. *B* shows a representative blot for TGF-α expression in control and DSS-treated mouse colon. The blot in *C* shows equal expression of ERK as control. (*D*) The graph represents densitometric analysis of 10 blots probing TGF-α expression in control versus DSS-treated mouse colonic mucosae. $*P < .05$; n = 10.

The data from the current studies suggest that EGF also partially restores ion transport capabilities, and particularly a sodium absorptive capacity, to inflamed colonic mucosa.

In various animal models of colitis and in patients with IBD, there is evidence that a number of ion transporters show decreased expression and/or activity in inflamed colon. Electrophysiologic studies have shown dramatic decreases in the absorptive capacity of inflamed colon, including channel-mediated apical Na^+ entry and Na^+,K^+–adenosine triphosphatase—mediated basolateral Na^+ extrusion.[4,20,22,36,37] Moreover, in the interleukin-2–deficient mouse model of colitis, the diarrhea that occurs is primarily due to decreased sodium uptake by ENaC in the absence of a barrier defect.[23] In our chronic model of colitis, we did not observe any damage to the

epithelium but did see decreased expression of α-ENaC, the amiloride-sensitive subunit of the heteromultimeric channel protein. This observation corresponds with data from patients with ulcerative colitis in which decreased expression of the α-ENaC subunit was seen in crypt but not surface epithelial cells.[20]

Electrophysiologic studies suggest that both elevated chloride secretion and reduced sodium absorption, along with decreased barrier function, contribute to the diarrhea and perturbed ion transport function of the colon in colitis.[4,22,37] We investigated the nature of the charge-carrying ion accounting for the modulatory effects of EGF on forskolin-induced ion transport in both normal and inflamed colon by performing ion substitution experiments. The inhibitory effect of EGF on forskolin responses in normal colon was dependent on both Cl^- and Na^+, suggesting that both chloride secretion and sodium absorption might be involved (Figure 3). However, we must also raise the caveat that these data can be interpreted as reflecting solely an effect of EGF on chloride secretion, as discussed previously. In contrast, in inflamed colon, substitution of chloride with isethionate did not prevent the potentiating effect of EGF on forskolin-stimulated PD responses, suggesting that electrogenic Cl^- secretion plays a secondary role, if any. Conversely, replacement of sodium with choline completely abolished the potentiating effect of EGF on responses to forskolin, indicating that Na^+ absorption is likely the principal ion transport event enhanced by EGF in the setting of colitis. This conclusion is additionally supported by experiments with the ENaC inhibitor amiloride. If sodium malabsorption is a primary factor in fluid retention in the colon, thus leading to diarrhea, then the Na^+ absorptive effects of EGF in inflamed colon likely contribute to the therapeutic potential of the EGF family of ligands. We did not investigate a possible role for HCO_3^- in the ion transport response to forskolin. However, if HCO_3^- is involved, then its role is probably a minor one because studies by Cuthbert et al[38] showed that secretion of HCO_3^-, which plays a secondary role to Cl^- secretion in the colon, is not an obligatory part of the ion transport response to forskolin in distal mouse colonic mucosa.

The integrity of the actin cytoskeleton has been shown to influence ion transport responses in cultured colonic epithelial cells.[24,25,39,40] We therefore investigated whether cytoskeletal integrity was involved in the potentiating effect of EGF on forskolin-stimulated PD responses. Cytochalasin D reduced the potentiating effect of EGF on ion transport responses to forskolin. These data may suggest that EGF stimulates shuttling of preformed vesicle-bound sodium channels from the cytoplasm to the apical membrane. Indeed, studies in Caco-2 colonic epithelial cells have shown that EGF can stimulate vesicular trafficking, resulting in increased insertion of other ion transporters into plasma membranes.[41]

Previous studies from our group have shown a key role for the ERK1/2 isoforms of MAPK in EGF-dependent negative regulation of chloride secretion in T_{84} colonic epithelial cells.[10,11] ERK activation also seems to play an important role in the ability of EGF to potentiate ion transport responses in colonic tissues from DSS-treated mice. Interestingly, pretreatment with PD98059 did not diminish the ability of EGF to inhibit forskolin-stimulated Cl^- secretion across healthy mouse colonic mucosa. This suggests that in addition to having opposing regulatory effects on ion transport responses in healthy versus inflamed colon, EGF selectively recruits different signaling pathways depending on the pathologic status of the colon. The mechanistic basis for this differential EGFR signaling in inflammation warrants investigation. Of note, however, pretreatment with PD98059 did attenuate responses to forskolin in tissues that were not exposed to EGF, suggesting that ERK activation is involved in cAMP-dependent ion transport events in healthy colonic mucosa. Interestingly, cytochalasin D has been shown to prevent activation of MEK and microtubule-associated ERK[42,43]; therefore, in our model, ERK activation may be cytoskeleton-dependent because cytochalasin D completely blocked the potentiating effect of EGF in inflamed colon.

Another key intracellular signaling effector that mediates effects of EGF on ion transport is PI3-K.[9,41,44–46] In our chronic DSS colitis model, PI3-K seems to be involved in EGF potentiation of ion transport because this effect was blocked by wortmannin. This supports other evidence that nonmitogenic effects of EGF, such as stimulation of Na^+ absorption or inhibition of Cl^- secretion in intact intestinal tissue, are mediated at least in part by increased PI3-K activity.[9,44] Interestingly, wortmannin blocked forskolin-stimulated ion transport but did not affect EGF inhibition of forskolin responses across normal mouse colon. These data suggest that differential recruitment of the PI3-K signaling pathway occurs in response to EGF or forskolin in normal versus inflamed colon.

Other possible downstream mediators of the regulatory effect of EGF on ion transport are the PKC family of serine/threonine kinases that have been implicated in a variety of cellular functions, including regulation of barrier function, membrane trafficking, migration, and cell differentiation.[47] In T_{84} cells, EGF inhibits chloride secretion via the sequential activation of PI3-K and the novel PKC isoform PKCε.[27] The novel and conventional

PKC isoform inhibitor Gö6850 also partially reversed the potentiating effect of EGF on forskolin-stimulated ion transport across colonic tissues from DSS-treated mice. These data suggest a role for PKC activation in the potentiating effect of EGF, presumably downstream of PI3-K, although the exact sequence of signaling events has not been investigated and we also do not have definite evidence of the PKC isoform(s) involved. In normal mouse colon, pretreatment with Gö6850 significantly inhibited forskolin-stimulated ion transport responses and reduced the inhibitory effect of EGF on forskolin responses, suggesting that PKC plays a role in the inhibitory effect of EGF in normal colon. The apparently conflicting results of PKC inhibition reducing forskolin-stimulated transport, but having some role in the inhibitory effect of EGF, may be due to differing PKC isoforms being involved in positive and negative regulation of ion transport events.[31,48] In regard to cellular targets of EGF, our studies suggest that subepithelial neurons may be involved in the potentiating effect of EGF on forskolin-stimulated ion transport responses because the neuronal blocker tetrodotoxin partly reversed the potentiating effect of EGF in inflamed colon. However, we have shown by immunohistochemistry that EGF activates epithelial PI3-K signaling, thus demonstrating that EGF added to the serosal side of inflamed colonic mucosa activates epithelial signaling pathways, which, in addition, supports our electrophysiologic findings that PI3-K mediates the potentiating effect of EGF on forskolin-stimulated ion transport across inflamed colon. Therefore, both the colonic epithelium and the subepithelial elements may be targeted by EGF. We have not investigated the relationship between subepithelial neurons targeted by EGF and colonic epithelial cells. One possible explanation may be through EGF activation of phospholipase A_2 leading to prostaglandin synthesis.[49] Phospholipase A_2 activation has been shown to influence ENaC activity in renal epithelial cells and can also activate PI3-K in muscle cells.[50,51]

An acute and temporary increase in EGFR expression has been observed in the trinitrobenzene sulfonic acid colitis model.[52] In our model, we observed an increase in crypt epithelial expression of the EGFR in inflamed colon. However, EGFR expression in control mouse colon was primarily detected in surface epithelial cells. Although this difference in EGFR distribution may be a reflection of EGFR involvement in epithelial repair, we cannot discount the possibility that it may also contribute to the differential effects of EGF on ion transport in control versus inflamed colon. The increase in TGF-α expression in colonic tissues from DSS-treated mice is intriguing and conceivably might contribute further to EGFR-mediated effects in inflamed tissue.

Therefore, our data are consistent with a scenario in which EGF activation of PI3-K recruits 2 separate cytoskeletal-dependent pathways, one of which causes an increase in the activity of Na^+ channels already present in the apical cell membrane via ERK and PKC activation,[53] while the other may result in increased Na^+ absorption via the insertion of preexisting channels/

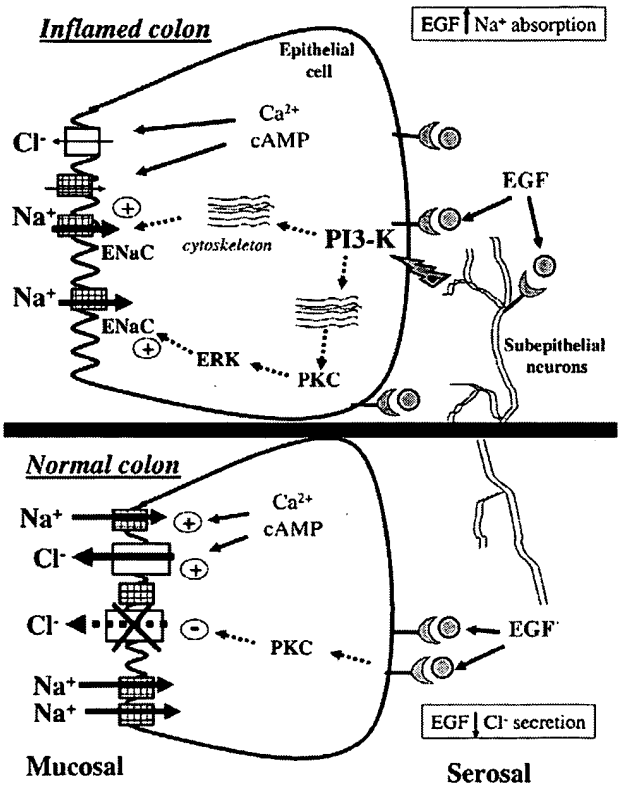

Figure 14. EGF potentiates ion transport responses in inflamed colon but inhibits responses in normal colon. In this schematic model, inflamed colonic mucosal tissues exhibit reduced ion transport responsiveness to cAMP and Ca^{2+} agonists (*thin solid arrows*). Pretreatment with EGF potentiates ion transport responsiveness by 2 pathways that are both PI3-K dependent and incorporate a cytoskeletal component. One pathway appears to be PKC and ERK dependent, whereas the other pathway is PKC and ERK independent (*broken arrows*). Both pathways converge at the level of the epithelial sodium channel (ENaC) in the mucosal membrane of the colonic epithelial cell to increase sodium absorption (*thick solid arrows*). A subepithelial neuronal component also appears to be involved, and this presumably increases PI3-K activation (*lightning bolt*). In normal colon, cAMP and Ca^{2+} agonists stimulate Cl^- secretion and Na^+ absorption. Pretreatment with EGF, acting independently of subepithelial neurons, inhibits ion transport responses by a mechanism that appears to involve PKC (*broken arrows*) but not ERK or PI3-K. Sodium and chloride channels are shown in the same cell for simplification of the model, although in vivo they are likely in different cells. The model also portrays the epithelial cell in inflamed colon as having fewer ENaC channels than the epithelial cell in normal colon, thus reflecting the observed decrease in α-ENaC expression in DSS-induced colitis.

transporters into the membrane or by increasing Na^+ channel activity in an ERK- and a PKC-independent manner (Figure 14). There is evidence in the literature that PKC activation can in turn activate ERK in a variety of cell types.[54,55] Indeed, activation of PI3-K can result in activation of both PKC and ERK, while activation of all 3 signaling species by EGF, resulting in modulation of ion transport and transporters, is well established.[9,10,27,44,46,56–58] This resulting increase in Na^+ channel activity and/or abundance then facilitates the electrogenic absorption of sodium. Sodium absorption is deficient in inflamed tissues due at least in part to decreased amounts of α-ENaC, as shown by immunohistochemistry. We should point out, however, that although α-ENaC levels were reduced in inflamed colonic epithelium, α-ENaC was still detectable in both the apical membrane and the cytoplasm of epithelial cells in inflamed colon. Membrane levels of ENaC could, however, be rate limiting for sodium absorption in inflamed tissue, allowing EGF to enhance this response by upregulating channel trafficking. EGF, via its effects on sodium transport, therefore can be considered to restore ionic homeostasis in the colonic mucosa in the setting of colitis.

In conclusion, in keeping with prior reports, we have shown that ion transport is abnormal in the setting of chronic colitis and is characterized by decreased sodium absorption. Moreover, EGF appears to partially restore agonist-stimulated sodium absorption in inflamed tissues, in contrast to its ability to inhibit active chloride secretion in the normal colon. The findings presented here may extend the mechanistic basis for observed beneficial effects of EGF and related growth factors in colitis. Additional delineation of the underlying signals may ultimately suggest new therapies for diarrheal conditions associated with IBD.

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